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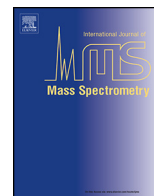
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Full Length Article

Native mass spectrometry imaging of intact proteins and protein complexes in thin tissue sections



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ABSTRACT

Here, we present native liquid extraction surface analysis (LESA) mass spectrometry imaging of proteins and protein complexes from mouse brain and liver tissue. Intact proteins were detected in characteristically low charge states, indicating that the proteins remain folded. In brain, abundant proteins such as ubiquitin and β thymosin 4 were detected homogeneously across the tissue whereas other proteins, such as neurogranin, were localised in specific anatomical regions. In liver, we demonstrate imaging of a protein complex (tetrameric hemoglobin), as well as fatty acid binding protein. Interestingly, the use of native-like solvents enables extraction of proteins which have not previously been observed in LESA experiments employing denaturing solvents, i.e., native LESA can be applied to extend the range of proteins observed. We also present native LESA ion mobility spectrometry and show that the collision cross sections of proteins extracted from tissue may be determined by travelling wave ion mobility spectrometry. The collision cross section of the 5+ ion of ubiquitin was calculated as 1047 Å², in good agreement with measurements of ubiquitin protein standard solutions. Collision cross sections for the 4+ ions of β -thymosin 4, β -thymosin 10 and two unidentified proteins were also calculated, together with that of a 10+ ion of an unidentified protein of molecular weight 15660 Da.

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1. Introduction

Native electrospray mass spectrometry (MS) has emerged as a powerful tool in the analysis of folded proteins and non-covalent protein complexes in the gas phase [1,2]. Non-covalent interactions are preserved through careful selection of non-denaturing electrospray buffers, and electric fields and pressures in the mass spectrometer [1,3–5]. As protein species remain in their folded state under these conditions, characteristically low charge states are detected owing to the limited availability of protonation sites.

The growth of native mass spectrometry has been accompanied by the emergence of ion mobility spectrometry, which enables the shape and conformation of the folded protein or protein complex to be probed [6]. Classical (drift tube) ion mobility devices use a uniform electric field to drive ions through a cell pressurized with a background gas. The drift-times of ionic species facilitate the measurement, from kinetic theory, of an absolute collision cross-section

(Ω) [7,8]. An alternative to the classic ion mobility method uses T-Wave-based technology to separate gas-phase ions (travelling wave ion mobility spectrometry, TWIMS) [9,10]. Collision cross sections can be determined from TWIMS measurements by calibration against ions of known Ω measured from drift tube measurements. Measurement of collision cross sections via TWIMS experiments allows derivation of information regarding molecular conformation [11–13].

Mass spectrometry imaging (MSI) enables spatial profiling of molecular species across a sample (such as a thin tissue section) providing insights into the co-localisation of different analytes and the relationship between the spatial distribution of specific molecules and tissue features. Mass spectrometry imaging of intact protein species directly from thin tissue sections is traditionally achieved via matrix-assisted laser desorption/ionisation (MALDI) [14]. Although the study of some non-covalent complexes has been reported via MALDI [15,16], it is only possible under very specific conditions. Recently, a range of alternative mass spectrometry imaging techniques have emerged which make use of electrospray ionisation (ESI). Desorption electrospray ionisation (DESI) [17–19] of proteins up to ~40 kDa from pure proteins spotted onto glass

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substrates has been demonstrated [18,20], however detection of proteins from tissue has not been reported to date. Liquid-DESI is a variant on this technique which has been described for the analysis of intact proteins and non-covalent protein complexes up to ~150 kDa [21], however only liquid samples can be probed in this way and so the technique does not lend itself to tissue analysis and/or imaging. Surface sampling techniques that couple to ESI based on liquid microjunction sampling have also been reported for protein analysis. These include liquid extraction surface analysis (LESA) [22–28] and continuous flow liquid microjunction sampling techniques, such as nanoDESI [29,30] and Flowprobe MS [31,32]. Each of these three techniques has been described for intact protein imaging using denaturing solvent systems [29,31–33].

As LESA mass spectrometry incorporates electrospray ionisation, it lends itself to native mass spectrometry, and we have recently demonstrated native LESA mass spectrometry of non-covalent protein complexes. Initial work focused on native LESA extraction of complexes of standard proteins (haemoglobin tetramers and holomyoglobin) from polymeric and glass substrates [34]. Native LESA mass spectrometry of larger protein assemblies (up to 800 kDa) and membrane protein assemblies from glass was subsequently demonstrated [35]. Alongside that work, we demonstrated native LESA mass spectrometry of haemoglobin tetramers from dried blood spots [34] and vasculature features within thin tissue sections [36], i.e., direct detection of protein complexes from physiological environments.

Here, we present native LESA mass spectrometry imaging of proteins and protein complexes in thin tissue sections of mouse brain and liver. As well as enabling detection of folded protein ions, the use of native-like solvents results in detection of proteins not previously observed in LESA experiments in which denaturing solvents were employed. We also present native LESA ion mobility spectrometry: collision cross sections for the 5+ ions of ubiquitin, 4+ ions of β -thymosin 4, 4+ ions of β -thymosin 10, and three unidentified protein ions, observed following native LESA sampling of mouse brain, were determined by use of travelling wave ion mobility spectrometry.

2. Materials and methods

2.1. Samples

Liver and brain from wild-type mice (extraneous tissue from culled animals) were the gift of Prof. Steve Watson (University of Birmingham). Organs were frozen on dry ice and then stored at -80°C until sectioned. The tissue was sectioned at a thickness of 10 μm using a CM1810 Cryostat (Leica Microsystems, Wetzlar, Germany) and thaw mounted onto glass slides. For calibration of TWIMS experiments, protein standards (ubiquitin, myoglobin and cytochrome c) and ammonium acetate were purchased from Sigma-Aldrich (Dorset, U.K.). HPLC grade methanol, water and acetic acid were purchased from Fisher (Loughborough, U.K.). Protein solutions were prepared at 10 μM concentration in 1:1 MeOH:H₂O with the addition of 2% acetic acid, 200 mM ammonium acetate buffer in H₂O or 200 mM ammonium acetate buffer in 5% MeOH.

2.2. Mass spectrometry and mass spectrometry imaging

Samples were introduced to the mass spectrometer via nanoESI using a Triversa Nanomate (Advion Biosciences, Ithaca, USA) coupled to a Synapt G2S mass spectrometer (Waters, Wilmslow, UK). The Synapt G2S mass spectrometer was fitted with a speedivalve (BOC Edwards, Crawley, Sussex, UK) between the rotary and turbo pumps that pump the ion source region, which enables the pressure in the source region to be controlled.

2.2.1. Direct infusion

A 15 μL sample of 10 μM protein solution in 49:49:2 MeOH:H₂O:acetic acid (for calibration standards in denaturing conditions) or 200 mM ammonium acetate in pure H₂O or 5% MeOH (for reference native ubiquitin collision cross section (CCS) measurements) was introduced to the mass spectrometer at a gas pressure of 0.3 PSI and 1.8 kV.

2.2.2. Surface sampling

Tissue sections on glass slides were mounted onto the LESA universal adaptor plate, and a digital image was acquired using an Epson Perfection V300 photo scanner. The exact location to be sampled was selected using the LESA Points software (Advion). The extraction/ionization solvent was 200 mM ammonium acetate in 5% methanol (HPLC grade, JT Baker, Deventer, The Netherlands). “Contact” LESA was performed as described in [28]. In the extraction process, 5 μL of solvent was aspirated from the solvent well before the robotic arm relocated above the tissue sample. The tip descended to a depth such that it was in contact with the tissue. Once in contact, 3 μL of solvent was dispensed and held in contact for 60 s before 3.5 μL of solvent was reaspirated and infused into the mass spectrometer. Samples were introduced to the mass spectrometer at a gas pressure of 0.15 PSI and 1.8 kV.

Mass spectra were acquired in the full scan TOF mode. The scan time was set between 2 and 5 s, and the m/z range was altered depending on the species investigated (usually ≥ 8000). The gas flow controls were optimised between 2–5 $\mu\text{L min}^{-1}$. All data were analysed by MassLynx software (version 4.1, Waters). The raw mass spectra have been averaged for 20–100 scans. For imaging experiments of mouse liver and mouse brain, data were acquired at 2 mm or 1 mm spacing respectively and for 2 min (60 \times 2 s scans) per pixel/location.

2.3. Travelling wave ion mobility spectrometry (TWIMS)

TWIMS experiments were performed on the Synapt G2-S mass spectrometer (Waters Corp., Wilmslow, UK) with the TWIMS ion guide maintained at 3 mbar of nitrogen. The following parameters were optimised for the ion mobility experiments using ubiquitin protein standards introduced by direct infusion electrospray ionisation: backing pressure (6.5 mbar), Trap/Transfer pressure (2×10^{-2} mbar), Trap collision energy (4 V), Transfer collision energy (2 V), Bias (3 V), Cone (150 V), IM DC entrance (20 V) and Transfer DC exit (15 V). TWIMS was operated at a wave velocity of 900 m/s and wave amplitudes of 40 V, 39.5 V, and 39 V. Collision cross section (CCS) calibration was performed following the procedure described by Ruotolo et al. [37]. Calibration standards included 7+ to 17+ charge states of cytochrome c, 8+ to 22+ charge states of myoglobin, and 9+ to 11+ charge states of ubiquitin, with CCS reference values obtained from Ruotolo et al. [37]. The normalised collision cross-section (Ω') values [$((\text{published } \Omega) \cdot \sqrt{\mu})/z$], where μ = reduced mass and z = charge], were plotted against corrected drift-time (t'_d) values of the calibrant ions measured at each wave amplitude. See Supplemental Fig. 1. Corrected drift times were calculated according to the equation $t'_d = t_d - \left[\frac{C\sqrt{m/z}}{1000} \right]$ where t_d is the experimental drift time in ms, m/z is the mass-to-charge ratio of the observed ions and C is the ‘EDC (Enhanced Duty Cycle) delay coefficient’, which on our instrument is equal to 1.43. Power regression of each plot to the form $\Omega' = At'_d{}^X$ gave the following: At wave height 40 V: $\Omega' = 321.88t'_d{}^{0.5508}$ ($R^2 = 0.9846$). At wave height 39.5 V: $\Omega' = 313.17t'_d{}^{0.5544}$ ($R^2 = 0.9844$). At wave height 39 V: $\Omega' = 312.98t'_d{}^{0.5493}$ ($R^2 = 0.9867$). The derived calibration coefficients were then used to calculate the collision cross-sections of the protein ions using the formula $\Omega = \frac{(At'_d{}^X)z}{\sqrt{\mu}}$.

where t_d' is the corrected drift time of the protein ion. CCS presented are the mean of values calculated at each wave height.

2.4. Data analysis

2.4.1. Mass spectra

Data were analysed using MassLynx version 4.1 software.

2.4.2. Imaging

Data files were loaded into MATLAB with imzML converter [38] using code adapted from Spectral Analysis software [39] downloaded from <https://github.com/AlanRace/SpectralAnalysis> on 21/03/2017 [39]. The spectra from multiple scans were summed at each location to form a single spectrum per position. Baseline correction and total ion count normalisation was performed on the summed data as described in the supplemental information (Supplemental File 1 and Supplemental Fig. 2). Peaks of interest were identified in MassLynx. The summed data were then used to create ion images of these peaks using in-house software (see Supplemental File 1).

3. Results

3.1. Native LESA of murine tissue sections

Increased pressure in the transfer region between the ion source and the mass analyser induces collisional cooling and desolvation of the ions, and has been demonstrated to be beneficial in both the native ESI of protein assemblies [40] and in the native LESA of pure protein complexes extracted from glass [41]. Fig. 1a shows typical native LESA-MS data acquired from mouse brain tissue at higher pressure (~6.5 mbar) and Supplemental Fig. 3 shows typical data acquired at lower pressure (3.5 mbar). A significant improvement in protein signal is observed, with signal-to-noise ratios for the multiply-charged protein peaks similar to those of unfolded singly-charged proteins previously reported in MALDI of untreated tissue [42].

Native LESA mass spectrometry of mouse brain tissue led to the detection of a number of protein species between 4 and 8 kDa, as shown in Fig. 1a. The abundant signals at m/z 1241 (4+, MW = 4960 Da) and 1713 (5+, MW = 8560 Da) can be attributed to β -thymosin 4 and ubiquitin respectively, both of which have been previously reported in LESA experiments using denaturing solvent systems [33]. In this work, ubiquitin was detected in 5+ and 6+ charge states. In contrast, ubiquitin extracted using denaturing solvent systems is typically detected in charge states between 8+ to 10+ [33]. Previous work by Gross et al. on direct ESI-MS of ubiquitin in ammonium acetate buffer solution also showed that the 5+ and 6+ charge states were the most abundant [43]. The lower charge states observed here are good evidence that the protein remains folded (fewer sites of protonation are available) [44].

The ability to profile proteins in their native state directly from tissue has important implications for future studies. The Synapt G2S mass spectrometer used in these experiments is a commercially available instrument equipped with travelling wave ion mobility spectrometry (TWIMS), which enables interrogation of protein conformation through measurement of collision cross sections. We applied TWIMS to the analysis of ubiquitin ions extracted from brain tissue by native LESA. The measured collision cross section (CCS) of the 5+ charge state of ubiquitin extracted from mouse brain via LESA in 200 mM ammonium acetate in 5% methanol is shown in Table 1. The mean value for the CCS was $1047 \pm 8 \text{ \AA}^2$, which is in good agreement with the measured CCS from the purified protein standard in both 200 mM ammonium acetate in 5% methanol ($1044 \pm 7 \text{ \AA}^2$) and 200 mM ammonium acetate in pure

Table 1

Collision cross sections of protein ions measured by travelling wave ion mobility spectrometry following native LESA of mouse brain.

m/z	Charge state	MW (Da)	Protein	CCS \AA^2 ^a
1713	5+	8560	Ubiquitin	1047 ± 8
1713	5+	8560	Ubiquitin ^b	1073 ± 16
1713	5+	8560	Ubiquitin ^c	1044 ± 7
1184	4+	4734	Unidentified protein	772 ± 5
1187	4+	4745	Unidentified protein	728 ± 6
1234	4+	4934	β thymosin 10	796 ± 2
1241	4+	4960	β thymosin 4	733 ± 2
1567	10+	15,660	Unidentified protein	2453 ± 17

^a Errors indicate one standard deviation above and below the mean value of three measurements at three different wave heights.

^b CCS measured via direct infusion native ESI of ubiquitin protein standard in 200 mM ammonium acetate.

^c CCS measured via direct infusion native ESI of ubiquitin protein standard in 200 mM ammonium acetate in 5% methanol.

water ($1073 \pm 16 \text{ \AA}^2$). (Quoted errors represent 1 standard deviation). Supplemental Fig. 4 shows arrival time distributions for the 5+ ions of ubiquitin extracted by LESA and in the two direct infusion ESI experiments. (Note that the peaks marked 1 and 2 are artefacts as confirmed by the extracted mass spectra at those times. See Supplemental Fig. 5). The collision cross section of ubiquitin in the 5+ charge state in helium, measured by drift tube ion mobility spectrometry, has been reported as 983 \AA^2 [45]. Our data were acquired via TWIMS in nitrogen gas: collision cross sections measured in nitrogen are larger than those measured in helium gas [6]. Ashcroft et al. [46] reported a mean CCS for the 4+ to 6+ charge states of ubiquitin of 1079 \AA^2 in nitrogen. Furthermore, they noted that the mobility peak for the 5+ ions was particularly broad, in agreement with our results.

Collision cross sections for a number of other protein ions extracted from brain tissue were also calculated, see Table 1. For example, the CCS of the 4+ ions of β -thymosin 4 (m/z 1241, 4960 Da) and β -thymosin 10 (m/z 1234, 4934 Da) were calculated to be $733 \pm 2 \text{ \AA}^2$ and $796 \pm 2 \text{ \AA}^2$ respectively. (To date, no literature values are available for comparison). It was also possible to calculate the CCS of a number of unidentified protein species; m/z 1187 (4+, 4745 Da), m/z 1184 (4+, 4734 Da) and m/z 1567 (10+, 15660 Da) were calculated to have CCS of $728 \pm 6 \text{ \AA}^2$, $772 \pm 5 \text{ \AA}^2$, and $2453 \pm 17 \text{ \AA}^2$ respectively. The mass spectral peaks (and signal-to-noise-ratios) of the ions for which CCS were calculated are shown in Supplemental Fig. 6.

Native LESA mass spectrometry of mouse liver tissue also led to the detection of a number of protein species, see Fig. 1b. Dominant signals at m/z 2042 (7+ charge state) and 1787 (8+ charge state) were attributed to a protein of molecular weight 14279 Da. That corresponds to the molecular mass of liver fatty acid binding protein (LAFABP), an abundant protein in liver tissue that has been previously reported and characterised by our group [25,47]. Typically, this protein is detected in charge states ranging from 12+ to 16+ under denaturing conditions (for example acetonitrile solutions containing formic acid). Similarly, protein species detected at m/z 1691 (5+ charge state) and 1409 (6+ charge state) (MW = 8445 Da), and 1653 (6+ charge state) and 1416 (7+ charge state) (MW = 9905 Da), correspond to the molecular masses of truncated ubiquitin (loss of two terminal glycine residues) and acyl Co-A binding protein, both of which have been previously reported in mouse liver tissue [33]. Ubiquitin (-GG) and acyl Co-A binding protein are usually detected in charge states ranging from 7+ to 8+ or 9+ to 11+ respectively. Again, the lower charge states detected here supports that the conclusion the proteins remain folded following LESA extraction. These results are a significant advancement on the methods reported in [36], in which no resolvable protein signals were detected in the bulk liver tissue.

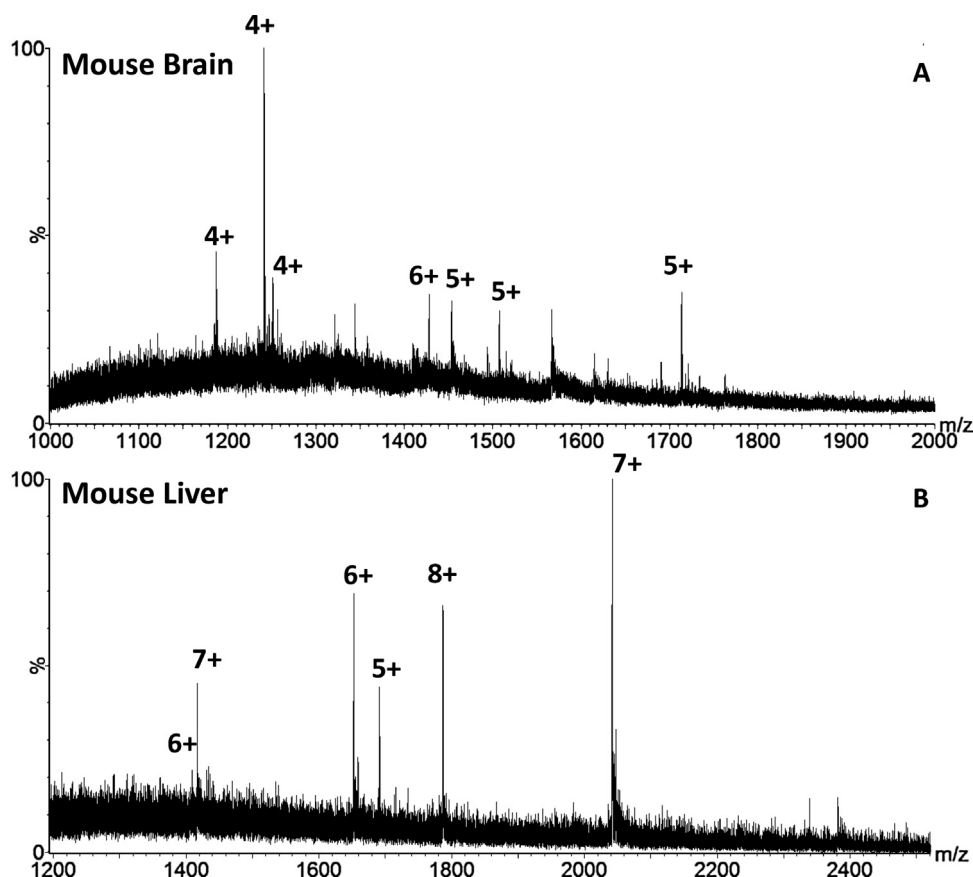


Fig. 1. Native LESA mass spectrometry of thin tissue sections of (A) mouse brain and (B) mouse liver tissue.

3.2. Native mass spectrometry imaging

3.2.1. Mouse brain

Fig. 2 shows ion images generated following native LESA mass spectrometry imaging of a sagittal section of mouse brain. (Details of data processing, including baseline subtraction and normalisation are given in the Supplemental information). Imaging was performed at 1 mm spacing which can be achieved without oversampling by contact LESA. (Currently, this is the smallest pixel size achievable by LESA-MSI due to software limits on spacing of sampling locations (1 mm)). Higher spatial resolution is achievable via other MSI techniques such as MALDI (typically $\sim 50\ \mu\text{m}$ lateral resolution); however MALDI is not suitable for the interrogation of folded proteins [16]. This spacing does however lead to long acquisition times (1 min sampling time, 2 min of data acquisition plus mechanical movement of the robot). In addition to this, nozzle blockage in the nanoESI chip can be particularly problematic when using sampling solvents containing ammonium acetate, hence it may be necessary to change nozzles before the data acquisition at each pixel can be started. The mouse brain imaging dataset presented here was acquired in approximately seven hours.

Some protein species were detected homogeneously across the tissue section, for example m/z 1241 (4+ charge state, 4960 Da, β -thymosin 4) and 1713 (5+ charge state, 8560 Da, ubiquitin), as shown in Fig. 2B and C. This is in agreement with previous LESA-MSI experiments using denaturing solvent systems [27,33]. Hsu et al. reported that β -thymosin 4 was detected in high abundance in the hippocampus and lower intensity in other brain regions via nanoDESI imaging [29]; however, the sagittal tissue section analysed in this study does not contain the hippocampus. Their study also showed that ubiquitin is homogeneously distributed in brain tissue, again in agreement with our results. Other proteins that

were homogeneously distributed were three unidentified proteins of masses 4745 Da, 12131 Da and 15660 Da, see Fig. 2D–F. Conversely, some protein species, such as acyl Co-A binding protein and truncated ubiquitin (Ub-GG), were particularly abundant in the cerebellum and brain stem regions, see Fig. 2G and H and Supplemental Fig. 7 for an annotated photo of brain regions. Acyl Co-A binding protein has been described in previous MALDI MS imaging experiments of coronal sections of mouse brain tissue [48]. Although not homogeneously distributed in their study, it is difficult to compare results to our data as the cerebellum was not studied in their experiment. Interestingly, truncated ubiquitin (Ub-GG), has a different spatial distribution to the intact ubiquitin. Hsu et al. observed truncated ubiquitin in their nanoDESI study of lymphoma thymus tissue sections; however it was not observed in healthy tissue [29].

A number of protein species detected in this experiment were not detected in previous LESA mass spectrometry imaging experiments performed with organic solvent systems, i.e., the results suggest that as well as maintaining tertiary structure, native LESA mass spectrometry increases the range of proteins detected. For example, an unidentified protein at m/z 1762 (8+ charge state, 14090 Da, see Supplemental Fig. 8A) was detected predominantly in the cerebellum and brain stem regions as shown in Fig. 2I. Neurogranin (7531 Da) (Fig. 2J, Supplemental Fig. 8B) was observed at m/z 1507 (5+ charge state), but has not been observed in previous LESA experiments with denaturing solvents, even with the incorporation of ion mobility separation [33]. Similarly, protein PEP19 (6718 Da) (Fig. 2K, Supplemental Fig. 8C) was observed at m/z 1344 (5+ charge state) and has not been identified by LESA-MS previously. Both of these proteins have been reported to be observed in the grey matter in MALDI imaging experiments of intact proteins. The spatial distribution of neurogranin is shown in Fig. 2J and appears to be

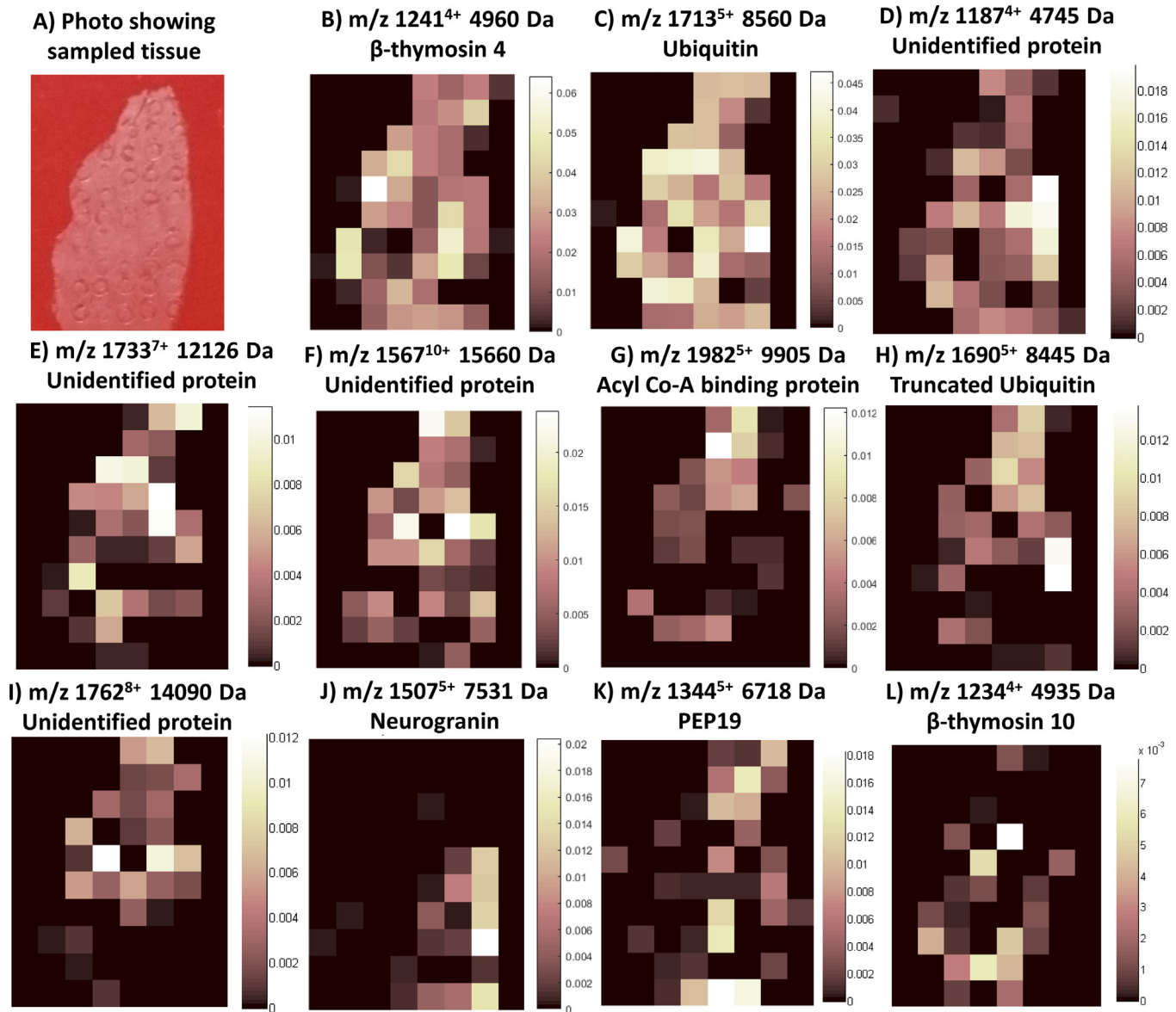


Fig. 2. Native LESA MS imaging of mouse brain. A scanned photo of the tissue after sampling is shown in panel A. Ion images show the spatial distributions of B) m/z 1241 (4+, 4960 Da, β thymosin 4), C) m/z 1713 (5+, 8560 Da, ubiquitin), D) m/z 1187 (4+, 4745 Da, unidentified protein), E) m/z 1733 (7+, 12126 Da, unidentified protein), F) m/z 1567 (10+, 15660 Da, unidentified protein), G) m/z 1982 (5+, 9905 Da, Acyl Co-A binding protein), H) m/z 1690 (5+, 8445 Da, truncated ubiquitin Ub-GG), I) m/z 1762 (8+, 14090 Da, unidentified protein), J) m/z 1507 (5+, 7531 Da, neurogranin), K) m/z 1344 (5+, 6718 Da, PEP19) and L) m/z 1234 (4+, 4935 Da, β -thymosin 10).

preferentially distributed in the cerebral cortex, in agreement with previous reports [49]. PEP19 was detected predominantly in the cerebellum. Previous studies considered coronal sections (no cerebellum) [49] hence it is difficult to compare results directly. The protein observed at m/z 1234 (4+ charge state, 4935 Da), (Fig. 2L and Supplemental Fig. 8D), appears to be distributed preferentially in the brain stem and the cerebral nuclei, however the distribution is less clear than for other ions as it was detected in relatively low abundance. This protein has been assigned as β thymosin 10 in previous top down LC MS experiments from extracts obtained from mouse brain tissue [48], however has not been reported in LESA-MS experiments to date.

3.2.2. Mouse liver

Mouse liver tissue sections were also imaged by native LESA mass spectrometry. Fig. 3A shows the mass spectrum acquired at a location corresponding to a visible vasculature feature and the corresponding spatial distribution of one of the ions detected

(m/z 3970). A photograph of the liver section and the LESA sampling array is shown inset. Peaks at $m/z \sim 3740$, 3970 and 4240 corresponding to the 17+, 16+ and 15+ charge states respectively of 64 kDa tetrameric haemoglobin were detected. Increased relative signal intensity of the haemoglobin tetramer complex at this location, as demonstrated in the ion image of the 16+ charge state, clearly demonstrates these methods lend themselves to mass spectrometry imaging of intact protein complexes. Tetrameric haemoglobin is the largest protein detected here following native LESA of tissue; however, it is worth noting that native LESA of the GroEL complex dried onto glass has been demonstrated [35] suggesting that there is no fundamental limit on molecular weight for native LESA, at least up to 800 kDa.

The spatial distribution of the 7+ charge state of liver fatty acid binding protein (LFABP) is shown in Fig. 3B. Conversely, this protein was present in greatest intensity in the bulk liver tissue and was not detected in the location of the vasculature feature. The spatial distributions of these ions correspond to similar experiments

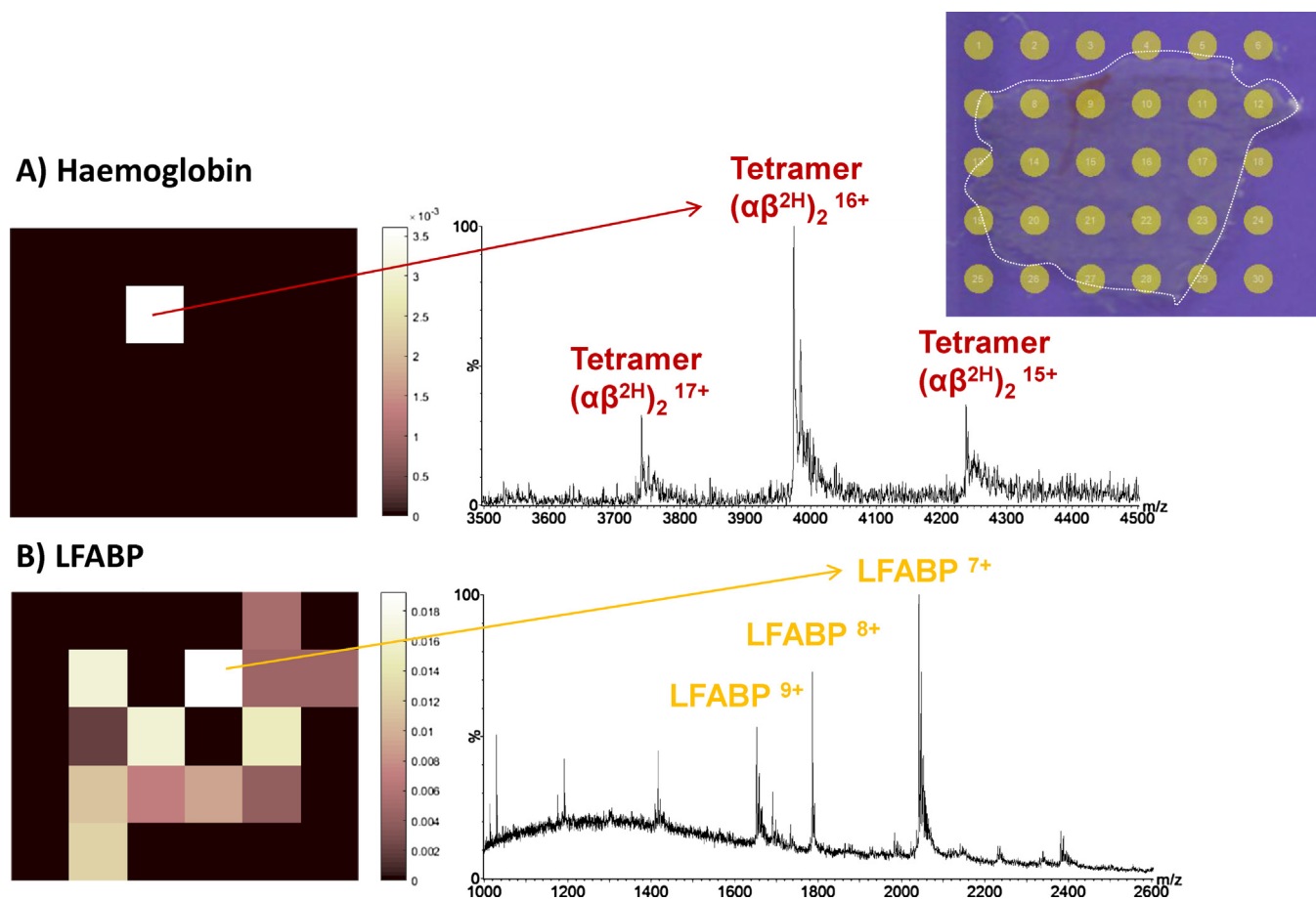


Fig. 3. Native LESA MS imaging of mouse liver. A) Ion image shown of m/z 3970 (16^+ , 64414 Da, $(\alpha\beta^{2H})_2$) and the mass spectrum acquired at location (2,3) showing detection of the haemoglobin tetramer in the 15^+ , 16^+ and 17^+ charge states. B) Ion image of m/z 2039 (7^+ , 14264 Da, LFABP) and the mass spectrum acquired at location (2,4) showing detection of LFABP in 7^+ , 8^+ and 9^+ charge states.

with denaturing solvents systems; the LFABP is homogeneously distributed across the bulk liver tissue and globin chains are highly abundant in the vasculature feature [33].

4. Conclusions

We have demonstrated native LESA mass spectrometry and native LESA mass spectrometry imaging of proteins directly from thin tissue sections. The low charge states observed are indicative of folded proteins. Our results show that use of native-like solvents enables extraction of proteins by LESA which have not been detected in previous experiments where organic solvent systems were employed. Moreover, native LESA mass spectrometry enables imaging of intact protein complexes (tetrameric hemoglobin). Lastly, we have shown that it is possible to measure collision cross sections of folded proteins extracted directly from tissue.

Native LESA mass spectrometry imaging and native LESA ion mobility spectrometry present exciting opportunities for the interrogation of protein-protein and protein-ligand interactions in a spatially-defined manner from biological samples. For example, these approaches have the potential to be applied to the study of protein misfolding in diseased tissue, protein-drug binding, or other protein-protein interactions implicated in disease.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ijms.2017.10.009>.

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